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GENOTYPES AND GENOTYPE × MEDIUM COMPOSITION INTERACTION EFFECTS ON ANDROGENETIC HAPLOID PRODUCTION IN CUCUMBER (*Cucumis sativus* L.)

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ABSTRACT

The present investigation was planned to determine factors affecting anther culture response traits in cucumber, especially growth hormone concentrations. Therefore, six cucumber cultivars, were used for anther culture technique. These cultivars were Poinsett 76, Telegraph Long, Speed Way, Algy, GY 14 A and Beta Alpha. Anthers from each cultivar were cultured on MS medium with three different levels of hormone balance. The data were recorded on each cultivar for responding anthers, calli and green point percentages. The obtained data were subjected to statistical analyses and the results could be summarized in the following: Highly significant differences were observed among genotypes for the three studied traits. Therefore, the planned comparisons between these genotypes as well as the further partition of phenotypic variance to its components are valid. The hormone balance levels as well as genotype × levels interaction mean squares were highly significant in both calli and green point percentage traits, but was not significant in the case of responding anthers. The cultivar 'Speed Way' was the best for all studied *in vitro* traits with respect to three hormone balances on MS medium.

In conclusion, the results could be recommended that each cucumber genotype or group of genotypes need altered media with suitable hormone balance for improvement their ability for haploid induction through anther culture technique.

INTRODUCTION

Since the first discovery of haploid plants in *Datura stramonium* (Blakeslee *et al*. 1922), haploids were induced from 247 species of angiosperms in 88 genera of 34 families (Maheshwari *et al*. 1983). Haploid plants have the gametophytic which have one-half of the normal number of chromosomes (Atanassov *et al*. 1995 and Zapata-Arias *et al*. 1995). These plants are of interest to plant breeders, which allow the expression of simple recessive genetic traits or mutated recessive genes and the doubledization of haploid could be used immediately as homozygous breeding lines (DH lines).

The most important factor prevents the use of haploids in cucumber (*Cucumis sativus* L.) breeding is the lack of an effective method for their production on a large scale. The development of an effective production system of doubled haploids and its further application in breeding programmes of cucurbitaceous crops could reduce the time required for cultivar development (Sauton, 1988). Anther culture and regeneration of plants from microspores greatly facilitate the subsequent recombinant selection in breeding programs. Moreover, anther culture technique reduce the time needed to reach homozygosity since either spontaneous or induced doubling of the haploid choromosome set results in homozygous and breeding true of diploid plants. In this respect, androgenesis induction through *in vitro* anther culture has been studied in cucumber (Lazarte and

Sasser 1982), Muskmelon and cucumber (Dryanovska and Ilieva 1983). All of them obtained haploid callus which yielded haploid plants at a very low frequency. Many factors affecting the success of anther culture process in most of crops, especially cucurbitaceae such as medium composition and growth conditions. In the case of *Cucumis melo***,** Metwally *et al.* (1998) reported a more effective method when cold pretreatment (4ºc for 4 days) male flower were put subsequently on the MS medium supplemented with 5 mg/l 2.4-D and 150 g/l sucrose. While, Shalaby (2007) studied genotype of donor plants effects on haploid production through *in vitro* ovule cultures among 12 genotypes of summer squash (*Cucurbita pepo* L.). They mentioned that genotype is a key factor influencing the *in vitro* gynogenesis in squash.

The purpose of this study was to investigate the factors affecting of anther culture response traits in cucumber (*Cucumis sativus* L.) and will be concentrated on the genotype effects of donor plants and media-composition effects as well as their interaction.

MATERIALS AND METHODS

In this investigation six cucumber cultivars belong to species *Cucumis sativus* L .were used for anther culture purpose. These cultivars were Poinsett 76, Telegraph Long, Speed Way, Algy, GY 14 A and Beta Alpha. MS medium with three concentrations of 2.4-D and BAP growth hormones were used as induction medium to produce haploid plants from six cultivars through anther culture technique. The medium composition was according to MS basal medium (Murashige and Skoog, 1962) containing 9% sucrose and three concentrations of 2.4 dichlorophenoxy acid (2.4-D) and benzyl amino purine (BAP). These concentrations were 1, 2 and 3 mg / l, respectively. However, the regeneration medium was MS containing 3% sucrose, and 1, 2 and 3 mg / l concentration of benzyl amino purine (BAP), in addition to 0.5, 1.0 and 1.5 mg / l naphthyl acetic acid (NAA). At the suitable stage of flower, when male buds having a length of 0.8 – 1.2 cm [Ashok Kumar *et. al.* (2003)] and containing anthers with mid to late uninucleat microspore stage were collected from 30 to 45 day – old plants. Buds for each cultivar were collected separately in small Petri dishes. These buds were kept at 4ºc for 1 – 4 days as cold pretreatment. Buds were sterilized by 1% HgCL2 for 20 minute. Then, washed three times with sterile distilled water. After that, the three anthers from each bud without filament were excide and plated on three 10 cm Petri dishes in diameter (each one with different growth hormone level) with induction medium. The dishes were incubated in the dark at 25ºc ± 2ºc for six weeks. Then, the produced calli and / or embryoids were calculated and transferred to regeneration medium for shoot development. The cultures were kept under 16 hours illumination at 22ºc ± 2ºc for 6 weeks. Subsequently, the green points were counted and transferred to MS regeneration medium (free hormone) for plantlets development. The experimental design was Randomized Complete Blocks design with four replications. Each replicate was represented by four dishes. Each Petri dish with 30-36 anthers was considered as experimental unit.

A combined analysis of variances for genotypes over the three hormone balances was made for the studied traits according to Steel and Torrie (1980). Subsequently, partition of phenotypic variance to its components were made for the studied *in vitro* traits: responding anthers, calli and green point percentages. In addition, heritability in broad sense was estimated according to the following equation:

$$\frac{\sigma^2 g + \sigma^2 gL/L}{\sigma^2 e + \sigma^2 gL/L + \sigma^2 L + \sigma^2 g} \times 100 = Hb\%$$

In order to normalize the data falls between 0.00 to 1.00, the percentage data for responding anthers and green point percentage traits were transformed by using $x^{1/2}$ arcsin prior to statistical analysis.

RESULTS AND DISCUSSION

The results of combined analyses of variance over the three levels of hormone balance for the studied traits are presented in Table 1. The genotypes mean squares were significant for responding anthers, calli and green point percentages. These finding indicated the presence of real differences among these genotypes with respect to the ability of anthers to response for anther culture purpose. Therefore, the partition of phenotypic variance to its components and the comparison between the genotypes means are valid. Furthermore, the hormone balance levels as well as genotype × levels interaction mean squares were highly significant in both calli and green point percentage traits. This results indicated that these genotypes gave different response at different hormone balance on MS medium. In this respect (Khatun *et al.* 2006) in eggplant and (Khanna and Raina 1998) in rice were reported that the performance of genotypes was different over the different media. This would be one possible way of avoiding the genotypic influence of regeneration ability by using various media depending on genotypic selection. Replications mean squares indicated the absence of significant differences between these genotypes for calli and green point percentage traits except for responding anthers trait which was highly significant. This finding indicated that the buds, which were collected from each genotype for anther culture purpose, are differed in their ability to anther culture.

Table 1: The analysis of variance of genotypes over the three levels of hormone balance for the studied traits

S.O.V	d.f	Responding anthers	Calli%	Green point%
Replications	3	598.66**	0.45	223.45
Genotypes (g)	5	1319.73**	1.75**	1060.05**
Levels (L)	2	438.99*	1.62**	2433.04**
g×L	10	176.16	1.17**	893.80**
Error	51	124.64	0.14	170.16

***,** are significant at 0.05 and 0.01 levels of probability, respectively.**

The data were transformed using arcsine $x^{1/2}$ for responding anthers and green point percentages prior to statistical analysis.

Note:

M1, M2 and M3 are MS medium supplemented with 2.4-D and BAP in three levels of 0.01, 0.02 and 0.03 gm/l as induction medium, BAP with three levels of 0.01, 0.02 and 0.03, gm/l and NAA with three levels of 0.005, 0.010 and 0.015 gm/l, respectively as regeneration medium.

Mean performance of the six cultivars over the three levels of hormone balance as well as their combined for the studied traits are presented Table 2. In the case of responding anthers, 'Poinsett 76' had the highest with mean of 72.35 with respect to M1 hormone balance response. Beside that, both 'Poinsett 76' and 'Speed Way' had the highest mean values to their response at M2 hormone balance with means of 64.06 and 68.46, respectively. They, also scored the highest mean values which were significantly higher than the other varieties with respect to M3 hormone balance with means of 76.47 and 79.95, respectively. While, 'Telegraph Long' scored the lowest mean value in comparison with other varieties on M1and M3 levels of hormone balance with means of 32.16 and 48.83, respectively. However, 'Telegraph Long' and 'Beta Alpha' had the lowest mean values for M2 level of hormone balance with means of 46.70 and 46.06, respectively. On the other hand, the comparison with combined data for responding anthers trait revealed that the highest mean values in both 'Poinsett 76' and 'Speed Way' with means of 70.96 and 68.08, respectively. However, the lowest mean value was observed also in 'Telegraph Long' with mean of 42.56.

In terms of calli percentage, the results showed that 'Poinsett 76' followed by 'Telegraph Long' had the highest mean values with means of 3.50 and 3.20, respectively in the case of M1 hormone balance. While, 'Speed Way' and 'GY 14 A' had the highest mean values which were significantly higher than the other varieties with means of 3.28 and 3.29, respectively. Moreover, the variety 'Speed Way' also had the highest and significantly differed than the other varieties with mean of 3.58 with respect to M3 hormone balance. In addition, 'GY 14 A' and 'Algy' scored the lowest mean values on M1 and M3 hormone balance with means of (2.64 and2.73) and (1.94 and 1.64), respectively. In addition, 'Beta Alpha' had the lowest mean value (1.47) in comparison with other varieties with respect to M2 hormone balance. The results which were collected from the combined data showed that, 'Speed Way' scored the highest mean value (3.28). However, 'Beta Alpha' and 'Algy' had the lowest mean values overall the other varieties with means of 2.20 and 2.37, respectively.

The collected data for green point percentage trait showed that 'Poinsett 76', 'Telegraph Long' and 'Speed Way' were the highest for regeneration ability on M1 hormone balance with means of 77.51, 78.40 and 71.51, respectively. While, 'Beta Alpha' had the lowest mean value (30.92) in comparison with other varieties. Only 'Speed Way' was the best regenerable variety on both M2 and M3 hormone balance with significant mean values with means of 66.84 and 85.89, respectively. Moreover, 'Telegraph Long' scored the lowest mean value 22.51 overall the other varieties for M2 hormone balance and 'Poinsett 76' scored the lowest mean value (47.75) for M3 hormone balance for the same trait. The results of combined data over the three hormone balance with respect to the highest and lowest regenerable ability varieties. The variety 'Speed Way' had the highest mean value (74.75) and significantly differed than the other varieties, while 'Beta Alpha' had the lowest mean value (48.75).

T2

This finding indicated that there were differences for the response of each genotype with different media which agree with (Kurtar *et al.* 1999 and Shalaby 2007) reported in Squash, (Suprunova and Shmykova 2008 and Niemirowicz-Szczytt *et al*. 2008) reported in Cucumber.

Genetic parameters for combined data over BAP and 2.4-D levels on MS medium is presented in Table 3. the results due to the combined data was subjected to determine the genotypic by hormone balances interaction ($\sigma^2 g \times L$) with respect MS medium was positive in the three studied traits. This finding may explain the different magnitude with different hormone balances. Also, the results of the combined data showed that the values of genotypic variance was positive, but less than environmental variance for the studied traits.

Table3: Genetic parameters from the combined data over the levels of hormone balance on MS medium

Genetic parameters	Responding anthers	Calli%	Green point%
$\sigma^2 Ph$	235.19	0.30	435.99
$\sigma^2 g$	95.30	0.05	13.86
$\sigma^2 L$	10.95	0.02	64.14
$\sigma^2 g \times L$	12.88	0.26	180.91
$\sigma^2 e$	124.64	0.14	170.16
Hb%	42.35	45.61	24.04
C.V	18.99 %	14.00 %	23.26 %

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تأثير التراكيب الوراثية والتفاعل بين التركيب الوراثي ومكونات البيئة المغذية على انتاج النباتات الاحادية من زراعة المتوك في الخيار

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تم عمل هذه الدراسة لتحديد العوامل التي تحد من الاستجابة لزراعة المتوك في الخيار وخاصة مستويات هرمونات النمو. من اجل هذا تم استخدام ٦ اصناف من الخيار هي Poinsett 76, Telegraph Long, Speed Way, Algy , GY 14 A بالاضافة الى Beta Alpha.

اخذت البراعم من كل صنف وتمت زراعتها على البيئة المغذية MS باستخدام ثلاثة مستويات من التوازن الهرموني. وتم تسجيل البيانات على ثلاثة صفات هامة بزراعة المتوك وهي: معدل الاستجابة لزراعة المتوك، معدل انتاج الكالوس ومعدل انتاج البقع الخضراء واستخدمت الطرق الاحصائية المناسبة لتحليل البيانات المتحصل عليها للتوصل للنتائج.

ويمكن ايجاز النتائج المتحصل عليها في الاتي : اظهرت نتائج اختبارات المعنوية لمتوسطات المربعات الى وجود اختلافات معنوية بين التراكيب الوراثية بالنسبة للصفات محل الدراسة مما يشير الى امكانية اجراء المقارنة بين هذه التراكيب الوراثية وتقسيم التباين المظهري الى مكوناته.

ايضا فانه في حالة اختبار المعنوية لمتوسطات المربعات بالنسبة لمستويات التوازن الهرموني و كذلك التفاعل بين التراكيب الوراثية × مستويات التوازن الهرموني الثلاثة ظهرت اختلافات عالية المعنوية بالنسبة لكلا من معدل انتاج الكالوس والبقع الخضراء ، بينما كانت غير معنوية بالنسبة لمعدل الاستجابة لزراعة المتوك.

كان الصنف 'Speed Way' هو الافضل بالنسبة لجميع الصفات المدروسة على البيئة المغذية MS باستخدام ثلاث مستويات من التوازن الهرموني مما يشير الى انه افضل التراكيب الوراثية تحت الدراسة لغرض زراعة المتوك في الخيار.

اظهرت النتائج ان لكل تركيب وراثي او مجموعة من التراكيب الوراثية في الخيار بيئة مغذية خاصة به بمستوى مناسب من التوازن الهرموني واللازم لتحسين قدرته على انتاج نباتات احادية من خلال تقنية زراعة المتوك.

قام بتحكيم البحث

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Table 2: Mean performance of the six cultivars over the three levels of hormone balance as well as their combined for the studied traits

Varieties	Responding%				Calli%				Green point%			
	M1	M2	M3	Comb	M1	M2	M3	Comb	M1	M2	M3	Comb
Poinsett76	72.35 a	64.06 a	76.47 a	70.96 a	3.50 a	2.53 b	2.38 bc	2.80 ab	77.51 a	40.31 b	47.75 b	55.19 b
Telegraph Long	32.16 b	46.70 b	48.83 c	42.56 c	3.20 ab	2.58 b	2.80 b	2.86 ab	78.40 a	22.51 c	60.23 b	53.71 b
Speed Way	55.84 a	68.46 a	79.95 a	68.08 ab	2.97 ab	3.28 a	3.58 a	3.28 a	71.51 a	66.84 a	85.89 a	74.75 a
Algy	59.55 a	58.71 ab	67.31 ab	61.85 ab	2.73 b	2.75 b	1.64 d	2.37 bc	57.42 a	45.15 b	54.47 b	52.35 b
GY 14 A	57.60 a	58.53 ab	53.51 bc	56.55 abc	2.64 b	3.29 a	1.94 cd	2.62 bc	52.13 ab	42.99 b	59.95 b	51.69 b
Beta Alpha	56.12 a	46.06 b	55.77 bc	52.65 bc	2.76 b	1.47 c	2.38 bc	2.20 c	30.92 b	49.00 b	66.34 ab	48.75 b
LSD 0.05	19.20	13.44	16.25	15.85	0.60	0.47	0.65	0.53	24.76	13.47	21.04	18.52

The means followed by the same letter in the same column are not significantly differed
The data were transformed using arcsine $x^{1/2}$ for responding anthers and plantlet percentages prior to statistical analysis.
Note:
M1, M2 and M3 are MS medium supplemented with 2.4-D and BAP in three levels of 0.01, 0.02 and 0.03 gm/l as induction medium, BAP with three levels of 0.01, 0.02 and 0.03, gm/l and NAA with three levels of 0.005, 0.010 and 0.015 gm/l, respectively as regeneration medium.